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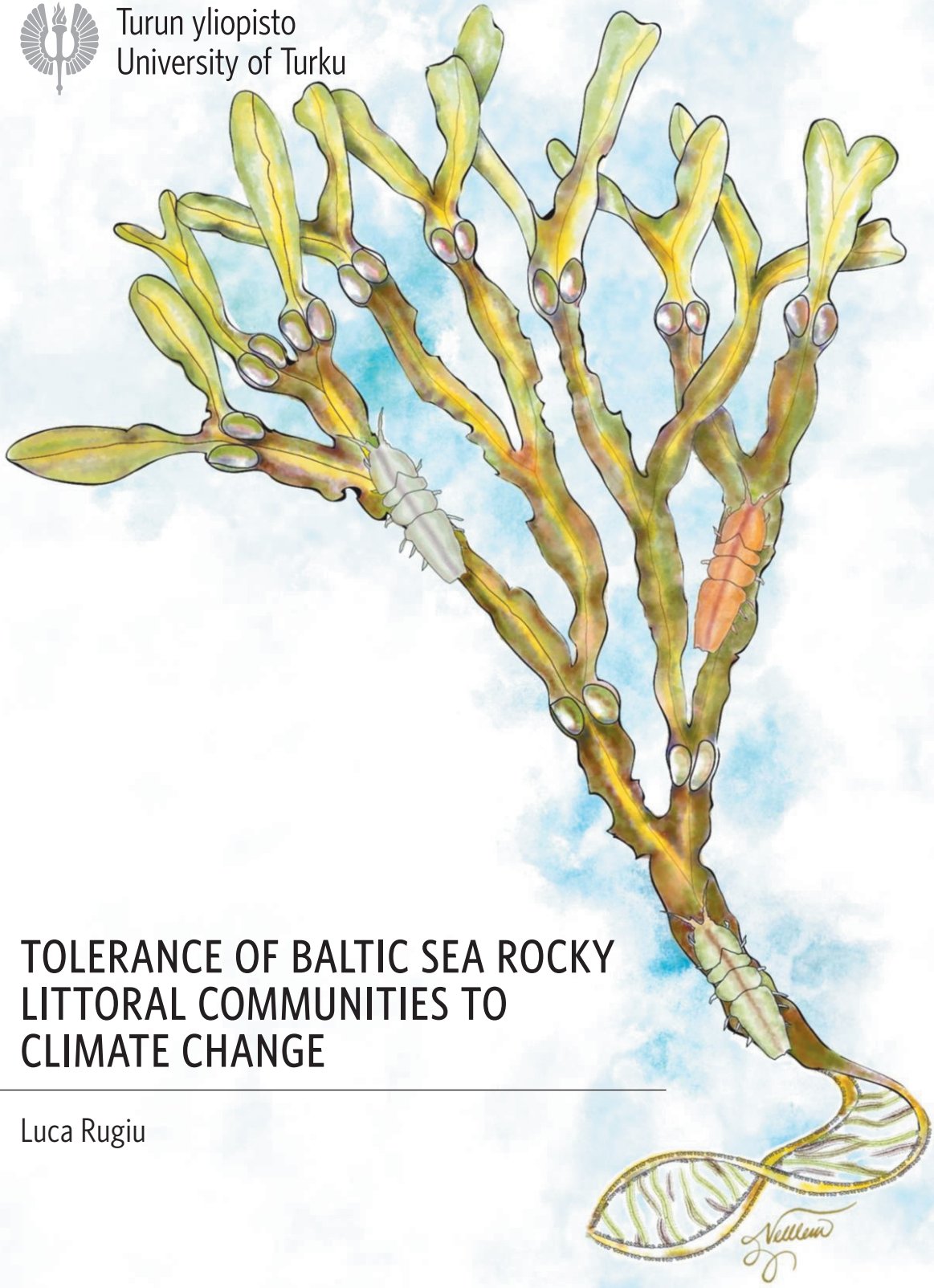

TOLERANCE OF BALTIC SEA ROCKY LITTORAL COMMUNITIES TO CLIMATE CHANGE

Luca Rugiu

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LIST OF ORIGINAL PAPERS

The present Ph.D. thesis is based on the following publications and manuscripts, which are referred to in the text by their Roman numerals.

I. Rugiu, L., Manninen, I., Rothäusler, E. & Jormalainen, V. 2018. Tolerance and potential for adaptation of a Baltic Sea rockweed under predicted climate change conditions. *Mar. Environ. Res. 134:76-84*

II. Rugiu, L., Manninen, I., Rothäusler, E. & Jormalainen, V. Tolerance to climate change of the clonally reproducing endemic Baltic seaweed: is phenotypic plasticity enough? *Fucus radicans. Manuscript*

III. Rugiu, L., Manninen, I., Sjöroos, J. & Jormalainen, V. 2018. Variations in tolerance to climate change in a key littoral herbivore. *Mar. Biol. 165:18*

IV. Rugiu, L., Panova, M., Pereyra R., Jormalainen, V. Populations of *F. vesiculosus* differ in gene expression response to low salinity. *Manuscript*

Author contributions to the original publication

	I	II	III	IV
Study design	VJ, LR, ER	VJ, LR, ER	VJ, LR, IM	LR, VJ, MP, RP
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Data analysis	LR, VJ	LR, VJ	LR, VJ, IM	LR, MP, RP
Writing	LR, VJ, IM, ER	LR, VJ, IM, ER	LR, VJ, IM	LR, MP, RP, VJ

LR = Luca Rugiu, IM = Iita Manninen, JS = Joakim Sjöroos, ER = Eva Rothäusler, MP = Marina Panova, RP = Ricardo Pereyra, VJ = Veijo Jormalainen. The order of initials indicates the relative contribution.

ABSTRACT

Climate change is challenging marine ecosystems worldwide, severely straining the tolerance of marine species and likely leading to distributional shifts. In the brackish-water Baltic Sea, there is a strong salinity gradient and pronounced seasonality, which together are responsible for its low biodiversity. These communities are dominated by very few species which fulfil the key ecosystem functions. Therefore, to predict how Baltic Sea communities might change in the future, it is first necessary to understand the effects of future changes on these key species, particularly with regard to their potential for adaptation.

I studied the consequences of future climate change, specifically in terms of simultaneous hyposalinity and warming, on three of the most important species in Baltic rocky littoral communities: *Fucus vesiculosus*, *Fucus radicans*, and *Idotea balthica*. Using indoor experiments, I exposed several populations of *F. vesiculosus* and *I. balthica* (from entrance, central, and marginal regions of the Baltic) and one population of *F. radicans* (marginal region) to both current ambient conditions and simulations of future climate (salinity and temperature). For both *Fucus* species, I replicated individuals in order to study variation within populations and within clonal lineages in tolerance to the future conditions. Furthermore, I analysed how short-term hyposalinity exposure affects gene expression in two populations of *F. vesiculosus*, to reveal the mechanisms behind acclimation to low salinity in this species.

The results of my thesis suggest that the effects of future conditions on *F. vesiculosus* and *I. balthica* will vary among and within Baltic regions. I found that hyposalinity and warming had the strongest effects on populations from the northern margin, as indicated by reductions in the survival and growth rate of *F. vesiculosus* and in the survival of *I. balthica*. These results may suggest that future conditions are likely to drive southward the distributional limits of *F. vesiculosus* and *I. balthica* in the Baltic Sea. Future conditions likewise hampered

the survival of *F. radicans*, but actually enhanced the growth rate of the survivors. I show that the most tolerant individuals of *F. radicans* may benefit from the future conditions, and thus the species is likely to maintain its distributional range and possibly even increase in abundance in the marginal region.

Furthermore, I found both among-population (*F. vesiculosus* and *I. balthica*) and within-population (both *Fucus* species) variation in tolerance to climate change, indicating the existence of genetic variation in plasticity with respect to future conditions. Marginal populations of *F. vesiculosus* also varied in gene expression when exposed to hyposalinity, although in general, the stress response to hyposaline conditions included an acute oxidative-stress response, inhibition of photosynthetic activity, and higher metabolic rate. Finally, I found that members of the same clonal lineage of *F. radicans* varied in their responses to the climate conditions. This result indicates that there may be variation in phenotypic plasticity within haplotype lineages in traits responsible for tolerance to environmental shifts, despite the putative lack of genetic variation. Standing genetic variation in phenotypic plasticity is an important component of adaptation, because it provides the variation upon which natural selection can act to pass on the successful traits to the next generation. Thus, this potential for adaptation may enable the future persistence of these key species, especially in the northern Baltic Sea.

TIIVISTELMA

Ilmaston lämpenemisen aiheuttamat muutokset meriekosysteemeissä koettelevat lajien sietokykyä ja muuttavat lajien levinneisyysalueita eri puolilla maailmaa. Itämeren murtovesiolosuhteissa eliöyhteisöjen monimuotoisuus on nykytilanteessa alhainen, sillä harvat lajit ovat sopeutuneet voimakkaaseen suolapitoisuusgradienttiin ja vuodenaikaisvaihteluun. Muutamat lajit ovat valta-asemassa, ja ne ovat ekosysteemin toiminnan kannalta ratkaisevassa roolissa. Jotta voidaan ennustaa ilmastonmuutoksen aiheuttamia vaikutuksia Itämeren eliöyhteisöissä, ensisijaisen tärkeää on selvittää, miten nämä avainlajit pystyvät sopeutumaan muuttuviin olosuhteisiin.

Väitöskirjassani tutkin kokeellisesti ilmastonmuutokseen liittyvän veden suolapitoisuuden alenemisen ja lämpötilan kohoamisen yhteisvaikutuksia Itämeren kalliorantojen valtalajeihin. Mallilajeinani olivat ruskolevät rakkolevä *Fucus vesiculosus* ja pikkuhauru *Fucus radicans* sekä äyriäinen leväsiira *Idotea balthica*. Kasvatin Itämeren etelä-, keski- ja pohjoisosien rakkolevä- ja leväsiirapopulaatioiden yksilöitä, sekä Itämeren pohjoisosien pikkuhauru yksilöitä vallitsevissa sekä tulevaisuudelle ennustetuissa veden suolapitoisuus- ja lämpötilaolosuhteissa. Tutkin populaatioiden välisen vaihtelun, ja yksilöiden välisen muuntelun määrää ruskolevien toleranssissa muuttuviin olosuhteisiin. Lisäksi tutkin geenien ilmentymisen muutoksia veden suolapitoisuuden muuttuessa kahdessa rakkoleväpopulaatiossa.

Havaitsin, että veden suolapitoisuuden alenemisen ja lämpötilan kohoamisen yhteisvaikutus tutkimuslajeihini vaihteli Itämeren eri osien välillä. Vaikutus oli suurin Itämeren pohjoisosan marginaalisissa populaatioissa, joissa rakkolevän ja leväsiiran eloonjääminen, ja rakkolevän kasvu oli heikompaa tulevaisuudelle ennustetuissa kuin vallitsevissa olosuhteissa. Tulokseni viittaavat siihen, että rakkolevän ja leväsiiran levinneisyysalueet siirtyvät Itämeren pohjoisosista etelämmäksi ilmastonmuutoksen edetessä. Tulevaisuudelle ennustetut suola- ja lämpötilaolosuhteet vähensivät pikkuhaurun eloonjäämistä, mutta paransivat hengissä selvinneiden yksiöiden kasvua. Sietokyvyltään parhaimmat yksilöt voivat näin ollen hyötyä ilmaston lämpenemisen aiheuttamista muutoksista, mikä voi mahdollistaa pikkuhaurun levinneisyysalueen säilymisen ennallaan.

Tutkimuksessani havaitsin myös populaatioiden välistä vaihtelua rakkolevän ja leväsiiran toleranssissa sekä populaatioiden sisäistä, yksilöiden välistä muuntelua ruskolevien toleranssissa tulevaisuuden olosuhteille. Veden suolapitoisuuden pienentyminen aiheutti rakkolevällä oksidatiivista stressiä, fotosynteesiin hidastumista ja aineenvaihdunnan kiihtymistä, mutta geenien ilmentyminen vaihteli yksilöiden välillä. Pikkuhaurulla vaste suolapitoisuuden muutokseen poikkesi samaa geneettistä alkuperää olevien ramettien välillä osoittaen, että toleranssin plastisuudessa on muuntelusta riippumatonta vaihtelua. Väitöskirjani osoittaa, että geneettinen muuntelu ruskolevien sietokyvyssä tulevaisuuden

olosuhteille voi mahdollistaa näiden lajien sopeutumisen ilmastonmuutoksen aiheuttamiin muutoksiin etenkin pohjoisen Itämeren populaatioissa.

1 INTRODUCTION

1.1 Climate change: the importance of temperature and salinity shifts

The synergistic impacts of natural climate variability and anthropogenic activities are affecting the physical properties of the planet (Harley et al. 2012; Vergés et al. 2014), and unprecedented changes are projected for the end of the 21st century (Kemp et al. 2015). The impact of climate change on ecosystem functioning is already evident, and it is reshaping biodiversity and species distributions worldwide (Bellard et al. 2012; Poloczanska et al. 2013; Lenoir and Svenning 2015). In the marine domain, these effects include shifts in the average regime and fluctuations in temperature and salinity, which are critical factors for biological communities.

The increased amount of atmospheric greenhouse gases and ozone stratification are the major drivers of global warming (Somero 2002; Harley et al. 2006; Sorte et al. 2011). Climate models suggest that the increase in mean temperature will be higher in high latitudes due to the decrease in ice albedo. In these regions, summer oceanic temperature may experience twice as much warming as in the rest of the world, while winter temperatures may warm up even quicker (Hansen et al. 2006; New et al. 2011). Global warming is not limited to a rise in average temperature, but will also result in extreme heat waves occurring with an unprecedented intensity (Frich et al. 2002). If water temperature exceeds a species' thermal limit of tolerance, it can have catastrophic consequences; these can even escalate to mass mortality events, as previously reported for gorgonians and sponges in the Mediterranean and Caribbean Seas (Lesser et al. 2007; Garrabou et al. 2009) and for *Mytilus edulis* in Northern Japan (Tsuchiya 1983). Such mass mortalities are still uncommon, but they represent just one of the effects of extreme warming. In most cases, seawater warming may be within the range of tolerance of marine species but

can still induce thermal stress (Williams et al. 2008). The resulting thermal stress produces physiological responses such as changes in energy metabolism or respiration which occur through intracellular adjustments of membrane composition, enzyme concentrations, and/or the production of heat shock proteins. Such changes may then have consequences for species fitness and population dynamics (Fields et al. 1993).

Besides warming, global climate change is also responsible for fluctuations in seawater salinity, both in terms of hypersalinity (Atlantic Ocean and Mediterranean Sea, Stott et al., 2008) and hyposalinity (Baltic Sea, Meier and Eilola, 2011). Water salinity defines the distribution of marine species in many coastal estuarine habitats, as well as in semi-enclosed basins such as the Baltic Sea (Snoeijs-Leijonmalm et al. 2017) and the Mediterranean Sea (Coll et al. 2010). Indeed, salinity is a major factor determining survival, growth, and reproduction in the marine domain (Qiu and Qian 1999; Normant and Lamprecht 2006; Łapucki and Normant 2008; Torres et al. 2011; Wrange et al. 2014). The osmotic tolerance of marine species depends on their ability to regulate their intracellular ionic composition and, in the case of photo-autotrophic species, to maintain a positive ratio of photosynthesis to respiration (Hellebust 1976). Despite its importance, variability in seawater salinity is little studied and the extent to which future salinity changes will impact marine communities is still poorly known (Boyer et al. 2005).

Future variations in seawater salinity and temperature are predicted to occur in concert, and their combined effect may have a stronger impact than that of only a single factor (Crain et al. 2009). Hence, studies aiming to estimate the effects of climate change on marine communities should carefully consider this synergy (Wernberg et al. 2012).

1.2 Responses of marine organisms to climate change

1.2.1 Potential for adaptation to future conditions

Understanding how marine organisms will react to future climate conditions is a challenging task, and species' success in the face of climate change may occur through different mechanisms. Many studies have demonstrated that species are able to respond to changing conditions by shifting their geographical distributions, which often happens by moving northwards (Perry et al. 2005; Harley et al. 2012; Poore et al. 2016; Knights et al. 2017). Interestingly, many of the abovementioned shifts were reported from intertidal communities, where the habitat is strongly influenced by both the water and atmospheric temperature.

A species may be able to cope with environmental change if the change remains within a tolerance range, and acclimation via short-term physiological responses allows individuals to persist (Bennett et al. 2015). However, if the environmental shift exceeds the tolerance range of a species, this will place selective pressure on the most-tolerant genotypes. Selection will be facilitated by high allelic richness and heritability in genes responsible for tolerance to the environmental change, which, in the long term, will enable the adaptation of the species to the new conditions (Pigliucci 2005; Merilä and Hoffmann 2016) . Selection may be further assisted by the presence of a high degree of phenotypic plasticity, i.e. the ability of a genotype to change its phenotype in response to the environment, which can increase the variety of traits upon which selection may act (Pigliucci et al. 2006).

The respective contributions of genotypic variation and phenotypic plasticity to the evolution of responses to climate change represent an ongoing discussion in the field of evolutionary biology. Some studies have hypothesised that phenotypic plasticity may negatively affect the rate of adaptation by inhibiting

selection for the best phenotypes (Ghalambor et al. 2007, 2015). Other studies have suggested that phenotypic plasticity is a product of environmental changes, and it may help the persistence of a species by buying time for evolutionary adaptation (Chevin et al. 2010).

The relative importance of genetic variation and phenotypic plasticity becomes very intriguing when it comes to species that reproduce both sexually and asexually. Indeed, for members of the same clonal lineage, a lack of genetic variation does not always reflect weak phenotypic variation (Lushai et al. 2003). Plastic responses to climate change have been already reported within clonal lineages of corals (Dubé et al. 2017b), macroalgae (Monro and Poore 2009), nematodes (Loxdale and Lushai 2003), and fish (Iguchi et al. 2001).

1.2.2 The importance of within-species variation in tolerance to climate change

Marine populations are often assumed to be highly connected to each other (Sanford and Kelly 2011), and the role of geographical and physical barriers in limiting the dispersal capability of marine organisms has generally been underestimated (Bertness et al. 2001). However, this view is being reconsidered in light of the fact that marine populations of a given species are often genetically and/or phenotypically different (Palumbi 2004; Levin 2006; Saada et al. 2016). Such within-species genetic structure can be a result of local adaptation or differences in tolerance among populations (Lamichhaney et al. 2012; Defaveri and Merilä 2014; Wrange et al. 2014; Muir et al. 2016) in addition to random processes such as genetic drift. This genetic structure may lead to differences in tolerance to environmental shifts, which makes it very challenging to predict a species' response to environmental change (Hereford 2009; Valladares et al. 2014). Thus, any estimation of the effect of future climate conditions on marine species should consider among-population variability, both that arising from geographical variation in environmental conditions and

that due to differences in population tolerance.

1.2.3 Experimental approaches for assessing the effects of climate change

Experiments involving the simulation of future conditions play an important role in testing the effects of future climate changes on marine organisms (Benton et al. 2007). This research has included the use of both indoor (e.g., aquaria or mesocosms) and field experiments that expose multiple individuals to different environmental conditions, such as current and projected future, and then compare responses among treatments. The inferences from these studies provide insights into the response of individuals to climate change after a relatively short period (Forsman et al. 2016). The experimental design defines how far the inference provided by the results can be generalised to natural populations. For this reason, factors such as the number of samples collected and the spatial scale of the sampling are crucial for the representativeness of the study. In general, this experimental approach yields estimates of the performance of current populations in future conditions, but it can also be used to estimate the potential for evolutionary processes like adaptation to future changes (Reusch 2014). Indeed, different populations and genotypes of known genetic background can be incorporated into experiments in order to understand the genetic contribution to variation in traits related to acclimation, thus testing for the presence of genetic variation in plasticity (Kawecki and Ebert 2004; Whitman and Agrawal 2009).

1.3 Aims of the research

The aim of my thesis was to test the effects of future climate conditions on Baltic Sea littoral communities by exposing three of the most dominant species to current and future simulations of salinity and temperature. For this, I chose

the brown algae and foundation species *Fucus vesiculosus* and *Fucus radicans* and the grazer *Idotea balthica* to investigate the effects of climate change on primary production and a major herbivore. As *F. vesiculosus* and *I. balthica* are widely distributed in the Baltic Sea, I designed the sampling scheme to include populations from throughout the entire Baltic Sea. Furthermore, I studied genetic variation and phenotypic plasticity in the tolerance of these species to the expected future salinity and temperature stress in order to estimate their potential to cope with the environmental change predicted for the Baltic Sea.

The aim of chapter **I** was to test the tolerance of *F. vesiculosus* to the future combination of decreased salinity and increased temperature expected to occur in 2070-2099 (Meier and Eilola 2011). I studied differences in tolerance to the future conditions among and within populations that inhabit different parts of the Baltic Sea. Such variation may indicate genetic variation in traits closely related to tolerance, which could provide the potential for adaptation to climate change. In Chapter **II,** I focused on *F. radicans*, and I tested the tolerance and potential for adaptation of a study population in the marginal region. Given the high level of clonality in *F. radicans*, I wanted to test the variation in performance both among different genotypes and within the same clonal lineage. In this way I aimed to measure the extent of phenotypic plasticity in this species in order to understand its potential for adaptation to future conditions. The experimental design in chapter **III** was similar to that of chapter **I**, but focused on *I. balthica*. Here, I explored variation in tolerance among replicated populations of isopods originating from different regions. Finally, in chapter **IV**, my goal was to describe the transcriptional adjustments behind the osmotic acclimation capability of *F. vesiculosus.* Here, I measured changes in gene expression in response to hyposalinity, and I tested whether populations varied in their acclimation responses to future salinity.

MATERIALS AND METHODS

1.4 The Baltic Sea

My study system is found in the Baltic Sea, a semi-enclosed body of brackish water situated between about 10-30°E and 54-66°N (Feistel et al. 2008). The combination of substantial inflow of freshwater from several rivers and limited seawater supply from the nearby Atlantic define a strong salinity gradient, starting from 20-25 PSU (Practical Salinity Units) in the Kattegat Sea (entrance), decreasing to 5-7 PSU in the Baltic proper (central region), and dropping to 2 PSU in the innermost part of the Bothnian Sea (marginal region) (Rönnberg and Bonsdorff 2004). Temperature likewise varies throughout the Baltic Sea, influenced by latitude and strong seasonality. Seasonality in particular has a high impact on the temperature of surface water layers. Indeed, surface seawater temperature may rise above 20°C in July, while in the coldest months, January and February, it may decrease to the freezing point (Swedish Environmental Protection Agency, 2001). The pronounced salinity gradients and seasonality are among the main determinants of the distribution and diversity of marine, brackish, and freshwater species in the Baltic Sea (Lass and Matthäus 2008).

Due to the low number of marine species present in the Baltic Sea, ecosystems tend to be dominated by only a few, which therefore fill crucial ecological roles and functions (Ojaveer et al. 2010). Even though Baltic marine species originated from the Atlantic Ocean, Baltic populations lost part of their genetic diversity during or after their establishment because of genetic drift and natural selection (Johannesson and André, 2006, Härkönen et al. 2005). This reduction in genotypic variation, combined with most species' limited dispersal capability, may promote phenomena such as population differentiation and local adaptation (Johannesson et al. 2011b). For species able to reproduce asexually,

the salinity gradient is an additional driver of reduced genetic diversity, as hyposalinity limits the optimal performance of gametes. Indeed, previous studies have demonstrated that seagrass and macroalgae reproduce mostly by fragmentation in the marginal region of the Baltic Sea (e.g., Bergstrom et al., 2005; Johannesson et al., 2011; Reusch et al., 2000; Tatarenkov et al., 2007). Due to this reduction in biological and genetic diversity, Baltic marine communities may be very susceptible to stressors such as climate change or other anthropogenic pressures.

1.5 The study species

I used three of the most dominant Baltic species to carry out four different manipulative experiments. *Fucus vesiculosus* (chapters I and IV) is the main foundation species of the rocky subtidal shores, from the Kattegat to the Bothnian Sea and the Gulf of Finland (Wikström and Kautsky 2007). This perennial macroalga is ecologically relevant for littoral communities because it supplies food and habitat to a large number of associated organisms (Lotze et al. 2001; Korpinen et al. 2007). Isolation by distance plays an important role in Baltic *F. vesiculosus* populations, with genetic differentiation being observed among populations even at a small scale (< 1 km) due to its limited dispersal capability. In the Bothnian Sea, clonal reproduction is common, further limiting the genotypic diversity of this alga (Johannesson et al. 2011).

Fucus radicans (chapter II) is the only endemic brown alga of the Baltic Sea, and its distribution is limited to the marginal regions, namely, the Bothnian Sea, Gulf of Finland, and Estonian coast (Forslund et al. 2012). In this part of the Baltic Sea, *F. radicans* shares the habitat with *F. vesiculosus*, from which it diverged ~ 400 years ago (Pereyra et al. 2009). *F. radicans* reproduces mostly sexually in the Estonian and Finnish coast (Pereyra et al. 2013; Ardehed et al. 2015), while vegetative reproduction dominates in the Western Bothnian Sea. Here, a few old

clones dominate algal populations, with some having spread over areas as large as 550 km^2 (Johannesson et al. 2011a, 2012). Some research suggests that the southern distributional limit of this macroalga is defined by a combination of the salinity gradient and grazing by *I. balthica* (Bergstrom et al. 2005; Gunnarsson and Berglund 2012).

Idotea balthica (chapter **III**) is among the most-dominant grazers in Baltic littoral communities, and its distribution overlaps that of *F. vesiculosus* (Leidenberger et al. 2012). The relevance of this isopod in the Baltic is linked to its abundance and the grazing pressure it places on micro- and macroalgae (Nilsson et al. 2004), and in particular on *F. vesiculosus*. Indeed, this grazer is able to consume up to 70% of *F. vesiculosus* biomass in one season (Haavisto and Jormalainen 2014). *I. balthica* also feeds on epiphytic ephemeral algae, and some studies suggest that this grazing activity can affect the competition for light and space between macroalgae and filamentous algae (Orav-Kotta and Kotta 2004). Since this species exerts top-down control on primary producers and serves as prey for numerous fish species, it has a key ecological role in energy transfer within Baltic marine communities (Leidenberger et al. 2012).

1.6 Sampling and experimental setups

1.6.1 The collection and preparation of samples for the experiments

In Chapter **I**, I aimed to study the tolerance of *F. vesiculosus* along the Baltic salinity gradient. To do this, I collected *F. vesiculosus* from three different populations within each Baltic region. Then, for the experiments in Chapter **II**, I sampled individuals of *F. radicans* from one marginal population (Fig. 1).

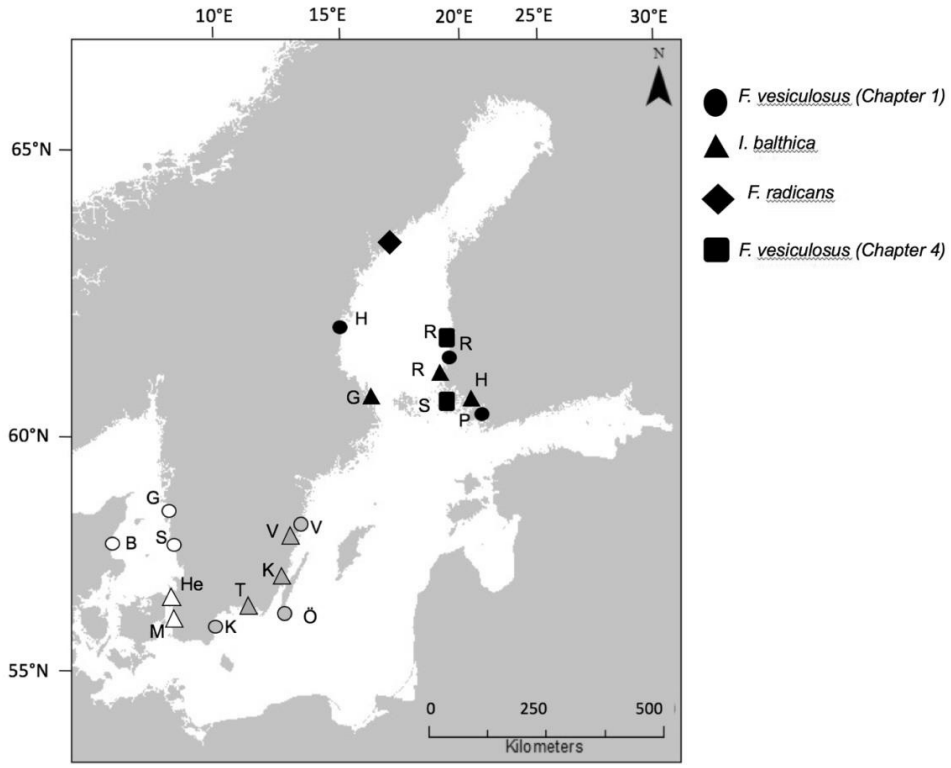

Figure 1. The Baltic Sea populations that were sampled for the studies in the thesis. Letters next to a symbol represent the code used for the population. Black symbols indicate sampling sites for marginal populations, grey symbols for central populations, and white symbols for the entrance populations.

I rinsed the algae of epiphytes, and split each thallus into eight branches of similar dimension. By replicating each genotype, I could measure its performance under different conditions. I randomly distributed four of these branches into aquaria set up to replicate the current salinity and temperature conditions in their home region, while the other four were placed in aquaria that replicated the future conditions expected for their home region (Table 1, Fig. 3). I kept *F. vesiculosus* from marginal regions and *F. radicans* in the same aquarium racks. I attached every alga to a tile with a cable tie to prevent flotation (Fig. 2a).

Area	Chapter I		Chapter II		Chapter III		Chapter IV	
	Current	Future	Current	Future	Current	Future	Current	Future
Marginal	5 PSU 14°C	2.5 PSU 16°C	5 PSU 14°C	2.5 PSU 16°C	5 PSU 11°C	2.5 PSU 14°C	5 PSU	2.5 PSU
Central	7 PSU 16°C	4 PSU 20°C			7 PSU 14°C	4 PSU 18°C		
Entrance	22 PSU 18°C	17 PSU 21°C			12 PSU 14°C	7 PSU 17°C		

Table 1. The current and future average summer sea surface temperature (°C) and salinity (PSU) used in the experiments of each chapter. Current conditions were calculated by averaging the monthly means from June to August of the sampling sites of each chapter, obtained from the Baltic Nest Institute (http://www.balticnest.org). The future expected conditions were set according to the model of Meier et al. (2012).

In chapter **III**, I sampled eight populations of *I. balthica* (Fig. 1), and from each population sampled 90 isopods. For the experiment, I placed each isopod into a transparent plastic tube that was closed with mesh over both openings to allow water flow and provided each animal with some *F. vesiculosus* as food and habitat (Fig. 2 -b). For this study, I exposed half of the samples from each population to their current region-specific salinity/temperature, and the other half to the future conditions (Fig. 3).

In chapter **IV**, I collected *F. vesiculosus* from two marginal populations (Fig. 1). In this case, I cut each thallus into two branches of similar size and prepared them for study in a way similar to that described for chapters **I** and **II.**

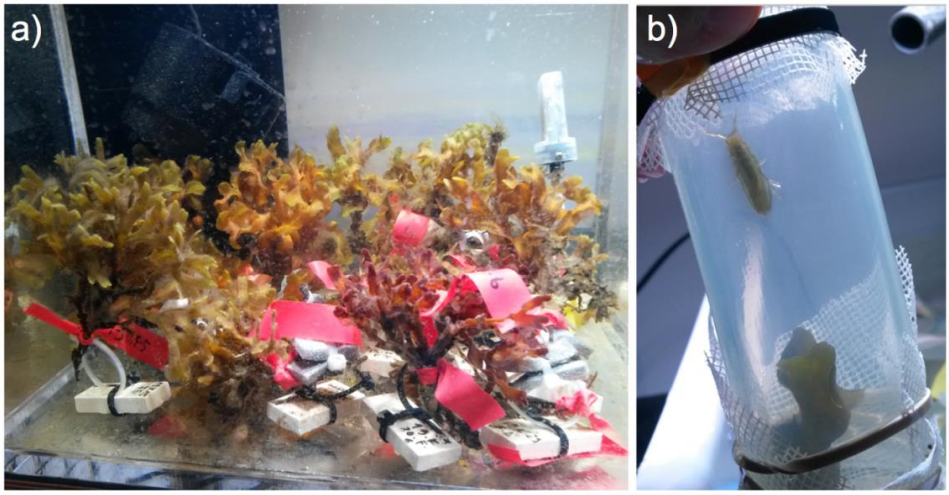

Figure 2 a) *F. vesiculosus* in an aquarium during the experiment (Chapter **I**).

b) An individual of *I. balthica* inside the container used in the experiment (in Chapter **III**).

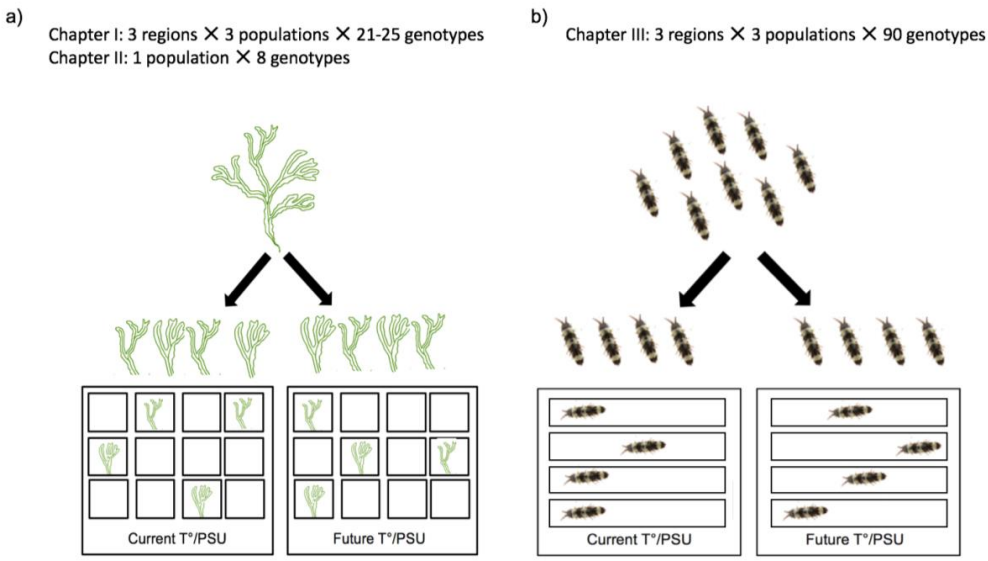

Figure 3. Schematic illustration of the experimental designs for a) *F. vesiculosus* and *F. radicans* and b) *I. balthica* in different studies of the thesis. Part (a) shows the splitting of each *Fucus* genotype into eight branches and their random placement in either current or future conditions. The number of populations and sampling regions varied

among the studies. Part (b) shows the random allocation of isopods from one population into aquaria in either current or future conditions.

1.6.2 The aquarium system for simulation of current and future conditions

I performed the indoor manipulative experiments at the Archipelago Research Institute (University of Turku, Finland) at Seili (60° 14′N, 21° 58′E). I used aquarium racks to expose each species to the current and projected future combination of summer salinity and temperature. I calculated the current summer conditions by averaging temperature and salinity from June to August (**I, II**), or June (**III**) from the data of the Baltic Nest Institute (http://www.baltic-nest.org). I set the current salinity for chapter **IV** according to *in situ* measurements due to the short duration of the experimental design (Table 1). I obtained the projected values for future salinity and seawater temperature for 2069-2099 separately for each Baltic region using the model RCAO-ECHAM-A2-REF from Meier and Eilola (2011). Salinity and temperature were manipulated simultaneously to mimic the natural environmental variability linked to climate change. I focused the studies on the summer conditions because during this time the growth of *Fucus* spp. is highest (Lehvo et al. 2001).

Each rack comprised a recirculating system in which the water was pumped from a bottom tank up to each aquarium and then flowed back by gravity. I used mechanical and biological filters to purify the water and a heater to control and modify the temperature. I supplied each shelf with two LED lamps and provided ample nutrient availability during the experiments. I made some adjustments to the design according to the experimental focus of each chapter. For chapters **I** and **III,** six racks were used, three for present and three for future conditions. The experiment for chapter **II** was conducted within two racks that were simultaneously being used for the experiment in chapter **I**. I ran the experiment for Chapter **IV** in two racks.

1.6.3 Experimental setup

For every experiment, I set up all racks first with current conditions. Then, after introducing all samples into their respective aquaria, I shifted the salinity and temperature of half of the racks to future conditions. For the studies in chapters **I** and **II**, I decreased salinity and increased temperature over the course of one day, and measured the growth and survival of the algae at the start and at the end of the experiment (140 days). I measured growth in terms of length of the main axis, wet weight, and the number of apical meristems. I expressed growth in terms of the rate of gain of biomass or length, and as the number of new meristems after 140 days. I also recorded the survival of the algae during the experiment, with an algal thallus demonstrating > 90% necrosis marked as dead.

For chapter **III**, I recorded the survival of each isopod every second day. For this study, I shifted the water conditions to the future conditions slowly over the course of 5 days, in order to allow the isopods to acclimate to the new conditions. I provided a longer acclimation period to the consumer than to the algae because isopods are usually able to move away from unfavourable conditions and may thus be less used to disturbance than macroalgae dwelling in high-disturbance environments. The duration of this experiment was 55 days, after which approximately 30% of isopods in future conditions were still alive.

For chapter **IV**, I shifted the salinity of the future rack over the course of three days, and exposed *F. vesiculosus* to the current and projected future salinity for 24 hours. At the end of the experiment, I cut the apical tips of each branch, immediately flash-froze them in liquid nitrogen, and stored them individually at -80°C.

1.7 Extraction of the genetic material

F. vesiculosus and *F. radicans* reproduce both sexually and asexually in the marginal region (Ardehed et al. 2016). With the aim of estimating the amount of among- and within-genotype variation in performance of these algae in future conditions (**I**, **II**), I needed to verify the number of clonal lineages included in my random sampling. For this, I extracted the DNA of the *F. vesiculosus* samples from the marginal region (chapter **I**: populations R and H) and the *F. radicans* population (**II**). I performed the DNA extraction according to the protocol described in (Johannesson et al. 2011a) and genotyped the thalli using ten microsatellites from Engel et al. (2003) and Perrin et al. (2007). I scored the number of genotypes using GeneMarker version 2.4 (SoftGenetics) and considered thalli to be clones when 100% of alleles matched. For the statistical analysis, I included the results derived from the genotyping; therefore the terms "genotype" and "clone" were based on this molecular analysis.

I extracted total RNA from *F. vesiculosus* (**IV**) using the protocol described in Pearson et al. (2006) and assessed the quality of the samples with a Bioanalyser and sequenced the 14 samples that passed the quality requirements for Illumina sequencing.

1.8 Statistical analysis

I analysed the growth and survival responses in chapters **I** and **II** with generalised linear models in SAS 9.4 (SAS Institute Inc. 2013). I used a Gaussian distribution and binomial distribution of the error variances, respectively, for growth rate (length, biomass and meristem gain after 140 days) and survival (dead/alive after 140 days). Since the current and simulated future conditions were averaged over the region of origin of the populations, I performed separate analyses of growth and survival within the entrance, central, and

marginal regions. Further, I set (**I**) population, genotype within population, and aquarium as random factors and climate change (two levels: current, future) as a fixed factor. For the analysis of *F. radicans* (**II**), genotype and aquarium were random factors; when a genotype included several ramets, I performed a separate analysis that included ramets within genotype and aquarium as random factors (a full description of algal genotyping is given in Supporting Information for the chapter **I**). I assessed the significance of the fixed effects using F-statistics and by estimating the denominator degrees of freedom according to Kenward and Roger (1997). I used the likelihood-ratio test (Littell et al. 2006) to derive statistical significance for the random effects and estimated individual performance (for both growth rate and survival probability) as the average of N=4 branches in the future and N=4 branches in the current conditions.

I analysed the survival of *I. balthica* after the 55-day experiment using the Cox proportional hazard model (Kalbfleisch and Prentice 2002) performed in SAS 9.4 (SAS Institute Inc., 2013). This regression analysis estimates the survival function with the Breslow method and tests the effect of the covariates on the survival rate with a Wald test. For this chapter, I set climate change, region, populations within a region, aquarium, and their interactions as covariates. For all analyses performed in chapters **I**, **II,** and **III**, I included all possible interactions between fixed and random factors at first. I then simplified the models using the Akaike Information Criterion (AIC) as a guideline and removed factors that did not improve the model fit, starting from the higher-order interactions

For the sequence analysis of gene expression in chapter **IV**, I filtered the sequences to produce the first full-length transcriptome of *F. vesiculosus* by *de novo* assembly in Trinity v. 2.3.2 (Grabherr et al. 2013). I retained only the reads supported by the read mapping and predicted the coding regions, then used the output of this for downstream applications. I analysed differences in gene expression between populations using principal components analysis in DESeq2,

which were visualised as a multidimensional-scaling (MDS) plot generated by ggplot2 (v. 2.2.1) (Wilkinson 2011) based on the read count. I tested the variation in gene expression between salinity conditions (current vs future), populations (Rauma vs. Parainen) and their combination with ANOSIM (analysis of similarity), executed with the vegan v. 2.0.3 package in R (Oksanen et al. 2007). Since this analysis revealed strong differences in gene expression among populations, I analysed the effect of hyposalinity separately for each population with a generalised linear model that used the normalised transcript count (factor salinity, two levels: current and future). The model was based on a negative binomial distribution and relied on Wald statistics. The results were adjusted for multiple testing using the false discovery rate (FDR) and a significance threshold of $\alpha < 0.05$. I conducted the annotation of the final transcriptome assembly by using Blastx to compare sequences to the NCBI nr protein database and by performing Gene Ontology (GO) mapping. Finally, I selected the most variant genes by retaining only those genes with a log2 fold change < -2 or > 2, and grouped them according to their putative function: response to oxidative stress, membrane/cytoskeleton composition and transport, and energy production and conversion.

RESULTS AND DISCUSSION

1.9 Spatial variability in tolerance to climate change

My results suggest that individuals of *F. vesiculosus* and *I. balthica* differ spatially in their tolerance to the future hyposalinity and warming projected for each Baltic region (Fig. 4). *F. vesiculosus* from the entrance region survived and grew equally well in both climate conditions, while the survival and growth rate of the central and marginal populations were strongly reduced (Fig. 4). Based on this, it appears that entrance populations may already be able to withstand the projected future shifts in salinity and temperature. In contrast, the expected abiotic shift may strongly affect the populations in the central and marginal regions, potentially constraining their future abundance and distribution. These results are consistent with those of Bäck et al. (1992b), who showed that acute osmotic stress caused a reduction in the growth rate and survival of *F. vesiculosus* from the marginal region. Likewise, this finding concords with that of Takolander et al. (2017), who reported that synchronous warming and hyposalinity stress not only damaged algal tissue and reduced photosynthetic efficiency but also reduced recovery from stress. The spatial gradient in tolerance to the projected climate conditions from the entrance to the marginal populations could be explained by adaptation to the local (regional) conditions. Indeed, although both central and marginal algae suffered when exposed to future conditions, the negative effects were stronger for those from marginal populations, where the future salinity will reach the lowest value predicted for the Baltic Sea (Fig. 4, 5).

F. radicans from the marginal region survived better in the future conditions than *F. vesiculosus* from the same region, and furthermore, the growth rate of the survivors was actually enhanced by future conditions (**II**, Fig. 4). This suggests that *F. radicans* may already be adapted to the future

hyposalinity/warming, and that it may, in fact, benefit from these conditions. Moderate warming can enhance algal metabolism and promote growth (Keser et al., 2005), and the increase in temperature in my experiment may be within the temperature range experienced by both of these species in nature. It is therefore possible that salinity might be the main factor responsible for the different tolerances of the two species. A reciprocal transplant study performed by Johannesson et al. (2017) indicated that *F. radicans* grows better when exposed to low salinity (4 PSU) than in the salinity of the Baltic Sea entrance (24 PSU), but that it can withstand the higher salinity better than *F. vesiculosus*. My observation of a difference in performance between *F. vesiculosus* and *F. radicans* likely arises from the fact that the latter species has a better ability to withstand osmotic stress.

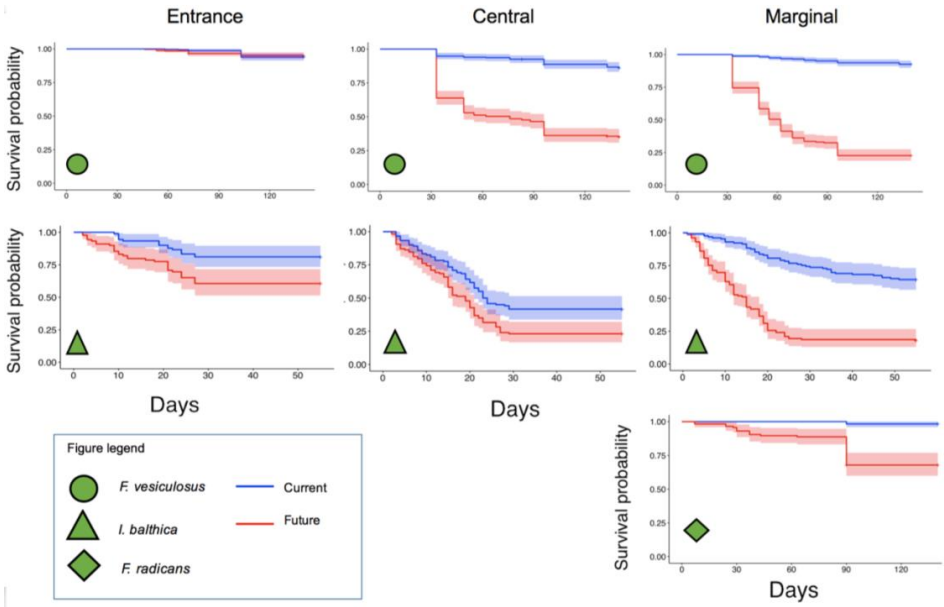

Figure 4 Survival curves with 95% confidence intervals for each population. Region of origin (entrance, central, or marginal region of the Baltic Sea) and the species studied are indicated for each curve.

The tolerance of *I. balthica* to future conditions showed spatial variation as well, with the marginal-region isopods having lower survival than the entrance-region ones in the future conditions. The average survival of the central-region isopods was low in both climate conditions, possibly due to sensitivity to handling during sampling or to the laboratory conditions. Therefore, any comparisons of the performance of these populations should be considered carefully (Fig. 4). The broad salinity and temperature tolerance of this species is considered to be one of the key features that have enabled its extensive distribution across the Baltic Sea (Leidenberger et al. 2012). However, acclimation to salinity (e.g., synthesis of sodium-potassium pumps) and temperature (e.g., modifying the balance between respiration and grazing activity) requires energy. The low tolerance of some populations may be explained by the high cost of acclimation to future conditions, which could lead to metabolic inefficiency and possibly to lower survival.

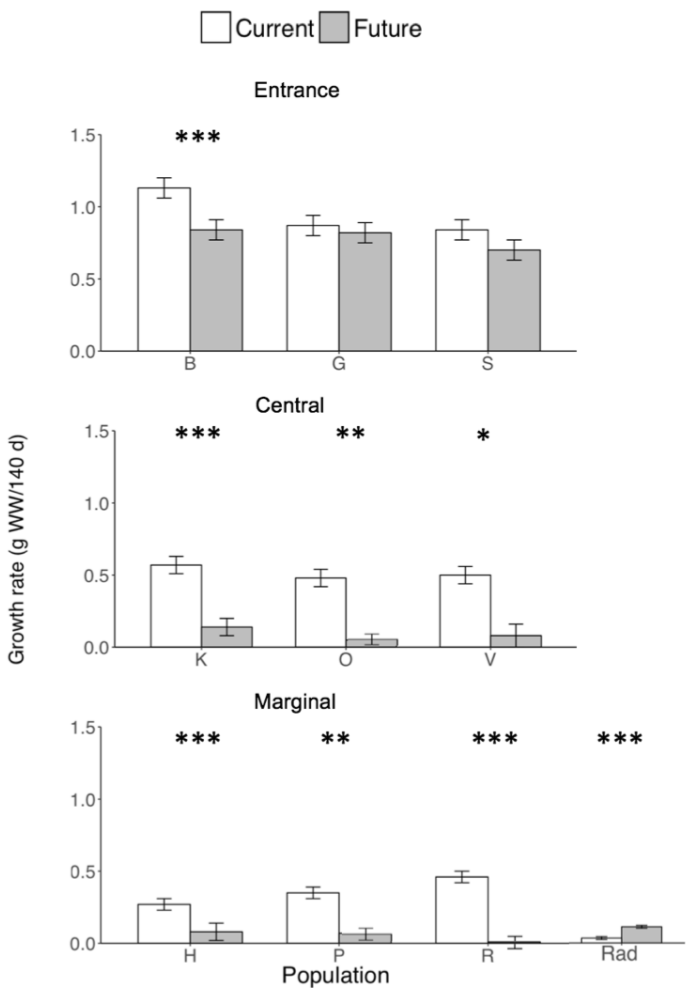

Figure 5 Growth rate (g WW/140d, mean ± SE, adjusted for the average initial size of algae) in current and future conditions for nine populations of *F. vesiculosus* and one of *F. radicans* (Rad) originating from three regions of the Baltic Sea. The number of genotypes (N) was 25 for each population except for H (N = 21), R (N = 23), and Rad (N=8). The identity of each population is indicated on the x-axis. Asterisks above denote the significance of the difference between results in current and future conditions (*p < .05, **p < .01, ***p < .001). Modified from Chapter **I.**

1.10 Potential distributional shift as response to future changes

My research indicates that future conditions will have little effect on the survival

and growth rate of *F. vesiculosus* in the entrance region. In contrast, the lower survival and growth rate found in the central and marginal populations suggest that the effects of a future environmental shift may be detrimental for these populations. Serrão et al. (1996) suggested that the distributional edge of *F. vesiculosus* is determined by the reduced sperm mobility and fertilisation success at salinities below 5 PSU. This limit for reproductive efficiency, together with the poor growth rate and survival found here, indicate that *F. vesiculosus* is likely to shift the northeast edge of its range to the south in the Baltic Sea. Instead, my results from chapter **II** show that most *F. radicans* individuals already have the ability to withstand future conditions, and, even if its overall survival decreases, the most tolerant genotypes will grow better, thus benefiting from the future conditions. If this happens, *F. radicans* might replace its congener as the main foundation species along the northern Baltic rocky shores.

Herbivory plays an important role in littoral communities, as it can seriously reduce the primary production of macrophytes (Poore et al. 2012). The high survival of isopods from the entrance populations in future conditions suggests that these populations will persist in this region, and their interaction with *F. vesiculosus* will be maintained in the future. However, the potential decrease in *I. balthica* abundance in marginal and central populations may have important ramifications for Baltic macrophytes. A reduction in grazing impact may be beneficial for ephemeral algae that compete with macrophytes for light and space. However, lowered herbivory could also contribute to the success of *F. radicans* in the marginal region. As shown by Forslund et al. (2012), *I. balthica* prefers *F. radicans* over *F. vesiculosus* as habitat and as a food source when both are available. If the distribution of this isopod shifts southwards, *F. radicans* may be released from this grazing control, and be able to take over the rocky habitat left abandoned by the possible distributional shift of *F. vesiculosus*.

However, it should be noted that the experimental manipulations I used, represents an extreme scenario of climate change. The future conditions

simulated in my experiments come from a modelled projection for the years 2070-2090, and the expected change in salinity and temperature will likely take place over several generations. This slower change, with respect to the time frame of my experiments, may provide enough time for adaptations to evolve via selection, which may be able to buffer the effect of the future environmental shift.

1.11 Among-population variation in tolerance to climate change

The variation in response to environmental stress among- and within-populations reflects, at least partly, the standing genetic variation within a species, which defines its potential to adapt to an environmental shift (Barrett and Schluter 2008). Several results of my thesis provide evidence that my target species host among- and/or within-population variation in tolerance to stress caused by future conditions.

For *F. vesiculosus*, entrance populations differed among themselves in their growth rate (**I**): future hyposalinity had no effect on two out of the three populations (Fig. 5). Among the marginal populations (**I**), two exhibited a higher growth rate than the third, even though the overall effect of the future conditions was negative (Fig. 5). A differing response between populations was observed also when two *F. vesiculosus* populations from the marginal region were exposed to a single type of salinity stress (**IV**). In this case, hyposalinity induced a change in the expression of only 26 genes in *F. vesiculosus* from one population, but 3072 genes in individuals from the other population (Fig. 6a and b). An analysis of the most-variant genes revealed that the performance of both populations in current conditions and of the R population in future conditions did not differ; instead, most of the variation was due to changes in the gene expression of the P population under hyposaline conditions (Fig. 7).

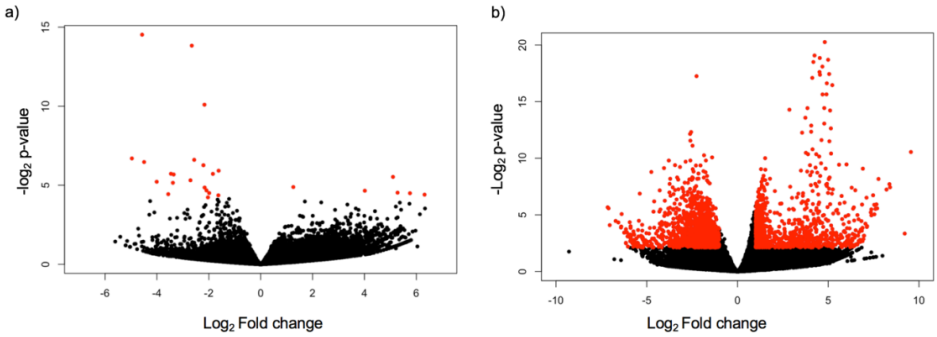

Figure 6. Graphical visualisation of the magnitude of differential expression of genes in response to hyposalinity for *F. vesiculosus* populations from a) Rauma and b) Parainen. Each point represents one of 33487 genes. The x-axis shows the fold-change and the y-axis shows the p-value for the contrast on a logarithmic scale. Upregulated genes are shown to the right of the plot, downregulated ones to the left. Significant changes (adjusted p-value < 0.05) are coloured in red.

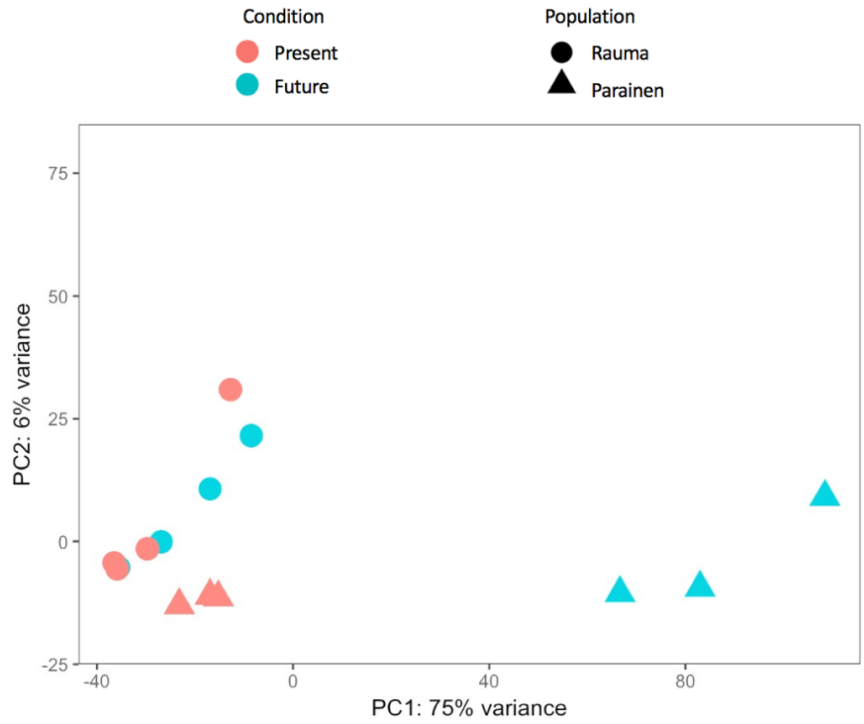

Figure 7 Multivariate ordination of *F. vesiculosus* samples based on the expression profiles of the most differentially expressed genes (FDR < -2 or > 2, P < 0.05). Grouping of the genes using principal components analysis was based on the normalized read

count from the differentially regulated genes, displayed for two populations and two salinity conditions.

My result from the survival analysis of *I. balthica* indicated that, also with this species, populations differed in their performance when exposed to future conditions (**III**). Out of two entrance populations, one population survived 45% less when exposed to future conditions, while in the other population survival remained unchanged in both conditions. Among the central populations, only one had a higher survival rate in current conditions. The other two populations suffered higher mortality in both current and future conditions, probably due to stress related to their sensitivity to manipulation. All marginal populations survived poorly in the future conditions.

The large-scale spatial variation in tolerance to future conditions found in *F. vesiculosus* and *I. balthica* could be caused by multiple factors. One hypothesis is that the tolerance of these species towards salinity and temperature extremes varies among Baltic regions. Alternatively, another explanation could be that the tolerance of these organisms does not differ spatially, but that their varying responses are due to the fact that I designed the climate conditions ad-hoc for each region. The existing literature tends to support the former hypothesis, as molecular studies have demonstrated that *F. vesiculosus* presents a high degree of genetic structuring within the Baltic Sea (Tatarenkov et al. 2007; Pereyra et al. 2013). This genetic differentiation among populations, combined with the strong environmental gradients of the Baltic Sea, make it more likely that my results were caused by adaptation to local conditions and different tolerance capabilities among populations of *F. vesiculosus*.

Although no studies have yet been performed on the genetic background of salinity tolerance for Baltic *I. balthica*, it is known that this isopod disperses by attaching itself to drifting algae or artificial material (Thiel and Gutow 2005) or by swimming relatively short distances. Earlier research showed that this species

tolerates a wide range of salinity and temperature conditions, and this feature allowed it to expand from the entrance to the marginal parts of the Baltic (Leidenberger et al. 2012). In addition, Wood et al. (2014) showed that populations of *I. balthica* from different Baltic regions are adapted to a wide range of salinity (from 10 PSU to 5 PSU). However, climate change may present a more significant challenge to the salinity tolerance of this isopod, as salinity is expected to drop below 5 PSU in the central and marginal regions (Meier and Eilola 2011). Such acute hyposalinity, in concert with warming, could produce a degree of stress that few populations are able to withstand, and this may be the reason for the among-population variation in tolerance that I found in chapter **III**.

The evidence of among-population variation in traits related to tolerance to a changing environment indicates that both *F. vesiculosus* and *I. balthica* may contain genetic variation and thus the potential to develop adaptations to future conditions. These findings underline the importance of including the possibility for geographical variation in tolerance when testing the effects of climate change on a species.

1.12 Among- and within-genotype variation in tolerance to climate change

The significant genotype-by-climate change effect in *F. vesiculosus* (chapter **I,** Fig. 8a) indicates that different genotypes differ in their responses to future conditions, thus providing evidence of genotypic variation in phenotypic plasticity for these traits. A substantial degree of genetic variation in phenotypic plasticity has been found for this macroalga in other studies. For instance, quantitative traits such as growth may vary in response to irradiance and nutrient availability (Jormalainen and Honkanen 2004), and phlorotannin production changes in response to low nutrients, herbivory, and depth

(Jormalainen and Ramsay 2009). The variation in response to climate change that I found may have important consequences for the persistence of the species, as the most-tolerant genotypes may be able to survive under the selection imposed by the future conditions and possibly pass their tolerance to future generations.

When genetic variation is lacking, phenotypic plasticity may replace it and generate the potential for adaptation (Chevin et al. 2010). In chapter **II**, 12 *F. radicans* thalli that belonged to a single clonal lineage varied in their growth responses to climate conditions, indicating that this lineage possesses a significant degree of phenotypic plasticity (Fig. 8b). Similarly, Johansson et al. (2017) found that members of a *F. radicans* clone varied in their growth responses to changes in salinity. Taken together, these two studies suggest that *F. radicans* may be able to rely on phenotypic plasticity to develop adaptations to future salinity and temperature conditions.

This intra-clonal variation is evidence that phenotypic plasticity may exist even when genotypic variation is lacking. One possible explanation for this outcome is that the genetic markers used were not informative enough to detect variation among members of the same clonal lineage. Indeed, genetic variation within clonal lineages is not uncommon in nature, and previous studies have found it in a diverse range of organisms (Dubé et al., 2017a; Lushai et al., 2003; Reusch et al., 1999). In *F. radicans*, somatic mutation during growth or during fragmentation of the thallus may be responsible for such variation. If this is the case, this somatic genetic variation may help this species by boosting its adaptive capability, as has been reported for a fire coral (Dubé et al., 2017b).

However, this within-clone variation may also be induced by the environment, through epigenetic changes that affect gene activity and its expression but do not cause direct changes in the genome (Joyce et al. 2003). Indeed, there is extensive evidence that clones of the same genotype can express genes differently as a result of epigenetic modifications (Caroline E Dubé et al., 2017b;

Evans et al., 2016; Mustonen et al., 2017; Ong-Abdullah et al., 2015; Perez et al., 2017). Although no studies have yet demonstrated the occurrence of epigenetic variation in seaweeds, it is certainly possible that this mechanism contributes to the within-clone variation in tolerance to climate change in *F. radicans*.

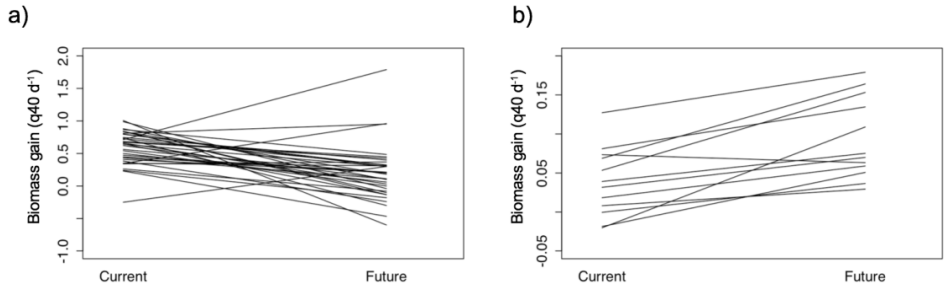

Figure 8 a) The variation in growth rate among genotypes in response to climate change for central populations of *F. vesiculosus*. Lines connect the average of each genotype (N=35) in the two conditions. The average for each genotype was calculated using 1 to 4 branches for each condition. Figure is replicated from chapter **I.**
b) Variation in growth rate for ramets of genotype A of *F. radicans* that were exposed to the current and future conditions. Each line represents the mean of a ramet, based on 4 replicated pieces of thallus reared in both current and future conditions. Figure is replicated from chapter **II.**

1.13 Physiological acclimation of *F. vesiculosus* to future hyposalinity

F. vesiculosus responded to hyposalinity with changes in gene expression, and these changes were particularly widespread in one of the two populations investigated (chapter **IV**). Exposure to hyposalinity induced the upregulation of at least 32 genes related to oxidative stress and osmotic balance, such as superoxide dismutase, disulfide isomerase, and nucleoredoxin-like proteins, which act to protect the cell from oxidative bursts caused by excess reactive oxygen species. Similar changes have been shown previously for other brown algae such as *Sargassum fusiforme* and *Ectocarpus siliculosus* (Dittami et al. 2012; Qian et al. 2016). Four genes involved in defence against oxidative stress

were downregulated; one of these encoded xanthine dehydrogenase, an enzyme responsible for osmotic adjustments which was reported to be upregulated in response to desiccation in *Arabidopsis spp*. (Yesbergenova et al. 2005). Since desiccation and hyposalinity affect algal performance in opposite ways, the downregulation of this gene that was observed here suggests that it may serve a similar function in osmotic adjustments for *F. vesiculosus*.

The cell membrane is the interface between the cell and the environment, and it is one of the most important cell components during osmotic stress. Exposure to the projected future salinity induced the upregulation of at least 16 genes and the downregulation of 31 genes related to membrane composition and transport. Genes related to the production of alginates and fatty acid composition were differentially expressed between salinity conditions, indicating their importance in hyposalinity adjustments and their role as important components of the brown algal cell wall (Coleman and Lee 2004; Deniaud-Bouët et al. 2014)

Fatty acids, in particular, are targets for reactive oxidative species (Zubia et al. 2007). The upregulation of their production that was observed here may thus serve to create replacements to maintain membrane stability. The regulation of ion transportation across the membrane was also highly affected by hyposalinity, with effects on genes encoding ATP-ase and voltage-gated ion channels; this pattern has been previously found in plants, red algae, and cyanobacteria as a response to osmotic pressure (Collén et al. 2007; Amtmann and Beilby 2010; Karsten 2012). The downregulation of such genes may be an acclimation strategy to balance the water potential of the cytoplasm through changing its intracellular ion composition.

F. vesiculosus inhibited its photosynthetic activity in response to hyposalinity by upregulating genes that produce carotenoids and control light harvesting (chapter **IV)**. Photosynthetic inhibition is a common reaction in photoautotrophs in response to osmotic, irradiance, and herbivory stress (Dittami et al. 2009;

Bilgin et al. 2010; Heinrich et al. 2012; Flöthe et al. 2014). This may promote acclimation for two main reasons. First, reduced investment in photosynthetic proteins may facilitate resource reallocation from primary to secondary metabolism. Second, because the photosynthetic electron transport chain generates oxidative damage due to the by-production of toxic oxygen derivatives, limiting primary production may also decrease the amount of reactive oxygen species (Niyogi 2000). While photoautotrophs are usually able to deal with these compounds, extra stress such as that caused by hyposalinity creates additional oxidative stress, which can be at least partially controlled by a reduction in photosynthetic activity.

The upregulation of genes related to the ATP cycle and respiration indicated an increased production of energy. For photoautotrophic species, the balance between respiration and photosynthetic activity is an important determinant of growth potential. An imbalance in the ratio of photosynthesis to respiration may lead to consequences that can result in lower growth or even, in extreme cases, to the death of the alga (Amsler 2008). Respiration also enables cells to process biochemical energy into ATP. The upregulation that was observed here could indicate that the algae were trying to fulfil an increased need for energy.

In contrast to the pattern observed for respiration-related genes, genes associated with carbohydrate metabolism were downregulated in response to hyposalinity, similar to what was found in *E. siliculosus* (Dittami et al. 2009). Photosynthetic activity strongly controls carbohydrate biosynthesis, and so this decrease may be directly correlated with the lower photosynthetic activity that was observed. However, carbohydrates are also used as osmolytes in response to salinity stress, and algae can accumulate these compounds in vacuoles or transport them out of the cell (Kirst 1989). Previous studies found that Baltic populations of *F. vesiculosus* differ from their Atlantic counterparts by having lower photosynthetic activity (Nygård and Ekelund 2006) and lower sugar content (Bäck et al., 1992). My findings indicate that the change in gene

expression related to these fitness components may not be solely a result of long-term evolutionary adaptation, but also an important part of hyposalinity acclimation in *F. vesiculosus*.

OUTLOOK AND FUTURE DIRECTIONS

In my thesis, I tested the tolerance to future climate conditions of two of the major foundation macrophytes of Baltic rocky littoral shores, as well as that of their most important herbivore. My experiments indicate that the effects of the projected warming and hyposalinity will vary among Baltic regions for *F. vesiculosus* and *I. balthica*. Specifically, the future conditions will have a stronger impact on central and marginal populations than on the entrance populations of both the host algae and the grazer. The poor performance of both these species in future conditions indicates that their abundance is likely to decrease in the central and marginal regions unless adaptation via selection occurs. I also showed that *F. radicans* may already be able to withstand the future warming/hyposalinity projected for the marginal region, and these conditions could even enhance its growth. Based on these findings, I hypothesise that future changes in salinity and temperature may lead to a distributional shift in the northernmost limit of *F. vesiculosus* and *I. balthica* in the Baltic Sea. Instead, *F. radicans* will most likely persist and possibly extend its distribution towards the central Baltic region, where the future climate conditions are projected to be similar to the current ones for the marginal region. This possible distributional shift may have major consequences for Baltic rocky littoral communities. Without *F. vesiculosus*, a high number of species in the marginal region may be deprived of food and shelter. Furthermore, the potential change in *I. balthica*'s distribution and abundance in some regions may decrease the strength of the producer-consumer link. Currently, this is a major determinant of ecosystem function, and a reduction in grazing pressure may lead to higher algal biomass (in terms of both ephemeral and macroalgal tissue), thus increasing the effect of eutrophication in littoral areas. In addition, a decrease in *I. balthica* abundance may reduce top-down control for epiphytic and filamentous algae, but may also have a bottom-up effect on coastal fish, as this isopod is an important part of their diet.

My thesis underlines the importance of within-species variation in response to environmental changes. Here, I discovered genetic variation in traits related to tolerance to future conditions both among populations (*F. vesiculosus* and *I. balthica*) and within populations (*F. vesiculosus* and *F. radicans*). Furthermore, for clonal *F. radicans*, I showed that a high degree of phenotypic plasticity may provide this species the potential for adaptation to future conditions. In light of these findings, predictive models of the effects of climate change on Baltic Sea species should consider spatial variation in species' responses and their potential for evolutionary adaptation.

Although my experiments yielded information relevant to the future of three key Baltic species, I could only focus on the response of a single species at a time. Marine communities are formed of multiple different species, and their structure and composition are defined by both abiotic and biotic factors, e.g., among-species competition and/or facilitation. A next step for research on climate change responses should include multiple species and should attempt to study how their performance will be affected by future conditions, as the biotic interactions may exacerbate, but possibly buffer, the effects of future environmental changes.

In the final chapter, I described how the gene expression of *F. vesiculosus* responded to the projected future salinity. This species undergoes major acclimation processes which involve changes in photosynthetic activity, energy metabolism, and membrane composition when exposed to lower salinity. In addition, I highlighted the importance of multiple genes related to the anti-oxidative response. This represents a step forward in our understanding of hypoosmotic regulation in this species. Finally, the among-population variation in gene expression to hyposalinity might yield clues to which regulatory regions of the genome are of particular interest for this species' potential for adaptation to future climate change.

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